

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C07C 279/24, 277/08, C07D 231/12	A1	(11) International Publication Number: WO 94/29269 (43) International Publication Date: 22 December 1994 (22.12.94)
(21) International Application Number: PCT/SE94/00517 (22) International Filing Date: 1 June 1994 (01.06.94) (30) Priority Data: 9301912-3 3 June 1993 (03.06.93) SE (71) Applicant (for all designated States except US): ASTRA AKTIEBOLAG [SE/SE]; S-151 85 Södertälje (SE). (72) Inventors; and (75) Inventors/Applicants (for US only): NYSTRÖM, Jan-Erik [SE/SE]; Ringstedsgatan 49, S-164 43 Kista (SE). SJÖBOM, Hans, Fredrik [SE/SE]; Kämpevägen 47, S-151 54 Södertälje (SE). (74) Agent: LARSSON, Birgitta; Astra Aktiebolag, Patent Dept., S-151 85 Södertälje (SE).		(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: PROCESS FOR THE PRODUCTION OF AMINOALKYLGUANIDINES (57) Abstract A process for the production of guanidino protected ω -aminoalkyl guanidines comprising reacting a diamine selectively at one amino group with an alkoxycarbonyl or aralkoxycarbonyl protected electrophilic guanylation reagent. The process of the invention is general and may be performed efficiently in one step yielding a product with high purity and in useful yields. The invention further refers to some compounds that are novel <i>per se</i> and to some novel compounds useful in the process of the invention.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Licchtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

PROCESS FOR THE PRODUCTION OF AMINOALKYLGUANIDINES

5 Field of the invention

The present invention relates to a process for the production of guanidino protected aminoalkyl guanidines, especially alkoxycarbonyl or aralkoxycarbonyl protected ω -aminoalkyl guanidines, such as N-t-butoxycarbonyl-N'-(ω -aminoalkyl) guanidines and N-benzyloxycarbonyl-N'-(ω -aminoalkyl) guanidines. Representatives of this
10 group of compounds are useful or potentially useful as peptide building blocks as mimics to the arginine residue. In particular the invention relates to a novel, one step process comprising reacting a diamine selectively at one amino-group with an alkoxycarbonyl or aralkoxycarbonyl protected electrophilic guanylation reagent. More specifically, the invention relates to a process reacting a symmetric 1,n-
15 diamino alkane with a alkoxycarbonyl or aralkoxycarbonyl isourea or isothiurea giving an guanidino protected ω -aminoalkyl guanidine. The invention further relates to some compounds that are novel compounds per se. These novel compounds were produced by using the process of the invention. Furthermore the invention relates to some novel compounds, useful in the process of the invention.

20

Background of the invention

Protected guanidines having an important role in the synthesis of peptides having arginine residues. Various mimics to arginine have been used as peptide building blocks. From a synthetical and commercial point of view, arginine-mimics having a
25 simplified structure compared to arginine are of particular interest. Perhaps the most attractive structural simplification of arginine is replacing the carboxylic group in arginine with a hydrogen giving a non-chiral molecule. The most important arginine derivatives in this context are noragmatine (N-(3-aminopropyl)-guanidine) and agmatine (N-(4-aminobutyl)-guanidine). More generally, ω -
30 aminoalkyl guanidines of varying chain length are interesting as peptide building blocks, particularly as a substitute for an arginine terminus in a peptide.

Previously known examples of applications of peptide building blocks, of the general type

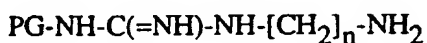
—NH-C(=NH)-NH-[CH₂]_n-NH— are, see for example:

US-A 4,387,049 disclosing example where n=3 (noragmatine), and

5 US-A 4,346,078 disclosing example where n=4 (agmatine).

A free guanidino group [—NH-C(=NH)-NH₂] provides synthetic complications and to be synthetically useful the building block requires a protective group (PG) which may be removed from the guanidino group at a desired stage of the

10 synthesis.



Frequently used protective groups for the guanidino group in arginine are for example alkoxycarbonyls such as t-butoxycarbonyl (Boc) and aralkoxycarbonyls such as benzyloxycarbonyl (Z). See for example: Wunsch E. "Methoden der

15 Organischen Chemie (Houben Weyl), Synthesen von Peptiden", 1974, 15/1, 506 ff. and Rzeszotarska Masiukiewicz Org. Prep. Proc. Int. 1988, 20, 427 ff.

The expressions Boc and Z are used in the following description as abbreviates for t-butoxycarbonyl and benzyloxycarbonyl, respectively.

20

Guanidino protected ω-aminoalkyl guanidines, PG₂-NH-C(=NH)-NH-[CH₂]_n-NH₂ (3), are generally prepared starting from a mono-protected diaminoalkane (1) in which the free amino group is reacted with an electrophilic guanylation reagent,

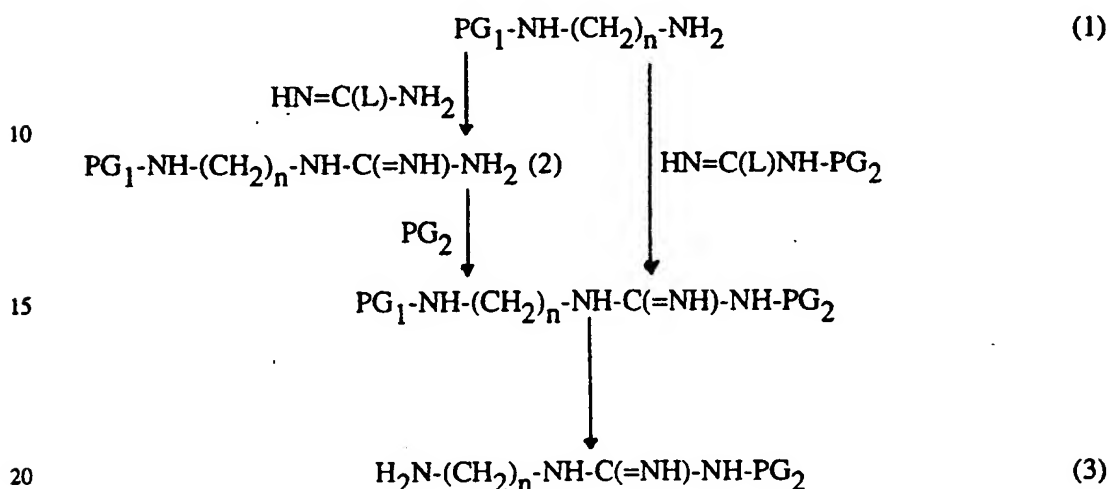
25 HN=C(L)-NH₂ where L is a leaving group, commonly used in guanidine syntheses. The reaction produces an amino protected ω-aminoalkyl guanidine (2) which after protection of the guanidino group with a second protective group (PG₂) and deprotection of the amino protective group (PG₁) gives the desired

PG₂-NH-C(=NH)-NH-[CH₂]_n-NH₂ (3). In this method the protective groups have

30 to be orthogonal. Examples of guanylation reagents that may be used are L= OMe, SMe, pyrazol-1-yl, 3,5-dimethyl-pyrazo-1-yl, and SO₃H. Recent developments have

proposed introduction of the second protective group, PG₂, in the guanylation reagent. Such a method simplifies the linear synthesis and shortens it with one step, (1 ⇒ 3), see scheme 1.

5 Scheme 1



Previously proposed guanylation reagents [PG₂-NH-C(L)=NH], carrying a protective group useful in guanidine syntheses, i.e. used to create a guanidino group, are disclosed in the following list:

25 for L/PG₂:

3,5-dimethyl-pyrazo-1-yl/NO₂ and 3,5-dimethyl-pyrazo-1-yl/tosyl; MeS/NO₂;

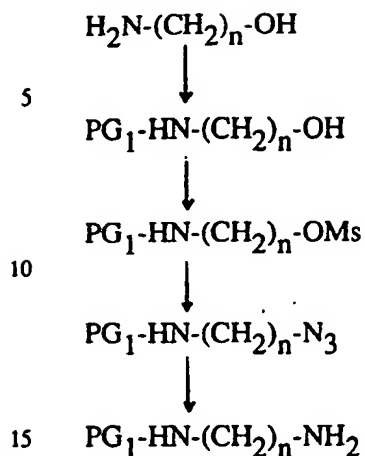
MeS/tosyl (See for example: Int. J. Peptide Res. 1991, 37, 425);

MeO/benzyloxycarbonyl (See for example: DE 3222342 A1);

30 MeS/benzyloxycarbonyl (See for example GB 2085444 A.

The mono protected diamine is generally prepared from the corresponding amino alcohol according to the four-steps protocol shown in scheme 2, (See for example Mattingly *Synthesis* 1990, 366).

35

Scheme 2

Consequently, methods previously known in the art totally require 6 to 7 synthetic steps in order to prepare a guanidino protected ω -aminoalkyl guanidine, $\text{PG}_2-\text{NH}-\text{C}(=\text{NH})-\text{NH}-[\text{CH}_2]_n-\text{NH}_2$ (3). See scheme 1 in combination with scheme 2. Such compounds, where the protective group (PG) is a alkoxycarbonyl or aralkoxycarbonyl, such as for example t-butoxycarbonyl or benzyloxycarbonyl, can now be produced more efficiently in the one step synthesis using the process of the present invention.

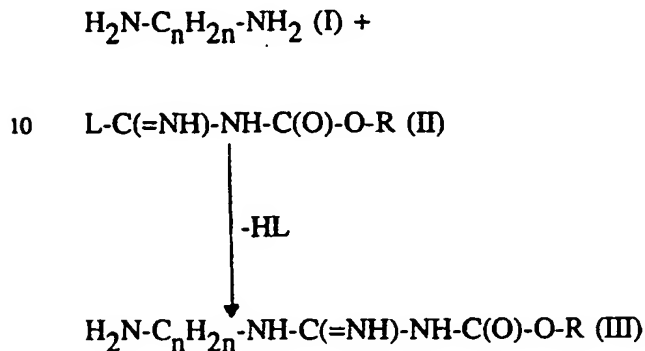
Brief description of the invention

The present invention may be considered as an improvement over processes known in the art for the preparation of aminoalkyl guanidines. Furthermore, the process of the invention contributes to the art of desymmetrization of symmetric substrates, more specifically to the art of desymmetrization of symmetric diamines. The present invention provides a novel and efficient process for the production of a guanidino protected ω -aminoalkyl guanidine of the general formula $\text{RO}-\text{C}(\text{O})-\text{NH}-\text{C}(=\text{NH})-\text{NH}-\text{C}_n\text{H}_{2n}-\text{NH}_2$ (III) or its tautomer $\text{R}-\text{O}-\text{C}(\text{O})-\text{N}=\text{C}(\text{NH}_2)-\text{NH}-\text{C}_n\text{H}_{2n}-\text{NH}_2$ or a salt of one of these, where R is an alkyl or aralkyl group and n is an integer 2 to 18. R is preferably a residue

promoting a crystalline product. More specifically, the process comprises reacting a symmetric primary 1,n-diaminoalkane (I) and a guanylation reagent (II) carrying a protective group yielding the protected ω -aminoalkyl guanidine (III) in one step, see scheme 3.

5

Scheme 3



15

Detailed description of the invention

The present invention is explained in detail in the following and especially by the accompanying examples. In a preferred embodiment of the process of the invention, the diamine (I) may be selected from 1,n-diamines of the general formula $\text{NH}_2-\text{C}_n\text{H}_{2n}-\text{NH}_2$, where $-\text{C}_n\text{H}_{2n}-$ is a linear or branched alkyl group and n is an integer 2-18, preferably a linear alkyl group where n is 2-12, and especially where n is 2-5, 8 or 12. The guanylation reagent may be selected from a group of compounds of the general formula $\text{ROC}(\text{O})-\text{N}=\text{C}(\text{L})-\text{NH}_2$ (II) or its tautomer $\text{ROC}(\text{O})-\text{NH}-\text{C}(\text{L})=\text{NH}$ or a salt of one of these. R is selected from the group consisting of a linear or branched C_1-C_{12} -alkyl group, preferably an alkyl group of 1-4 carbons such as methyl, ethyl, linear or branched propyl and butyl, preferably t-butyl, and an aralkyl group such as benzyl or substituted benzyl, preferably benzyl. L is a leaving group, which is useful in a guanylation reaction such as R^2O , R^2S , pyrazolyl, and substituted pyrazolyl, preferably 3,5-dimethyl-pyrazolyl, where R^2 is a lower alkyl, preferably a linear alkyl chain with 1-4 carbon atoms,

30

methyl and ethyl are preferred.

The reaction may be performed in the presence or in the absence of a solvent, preferably the reaction is performed in the absence of a solvent. If a solvent is used
5 said solvent can be: an aromatic hydrocarbon, such as an alkylbenzene and more specifically toluene or xylene; a hydrocarbon being linear or branched, cyclic or acyclic such as hexane, heptane, or cyclohexane; an alkyl nitril such as acetonitril; an alcohol such as isopropanol; or water.

10 There are two amino groups in the diamine, which may be guanylated. To suppress the side reaction where both amino groups are guanylated, the process is performed with about at least a stoichiometric amount of the diamine reactant in relation to the guanylation reagent, preferably employing an excess of the diamine reactant. The reaction can be performed with about 1-10 mol-equivalents, or preferably 1.5-6
15 equivalents, or most preferably 2-4 equivalents of the diamine reactant in relation to the guanylation reagent.

According to the process of the invention, the reactants and the solvent can be added to the reaction vessel in an arbitrary order at a suitable temperature,
20 preferably the reactants and optionally the solvent(s) are mixed at ambient temperature. The reaction may be performed at about 20-80°C, preferably at 40-60°C. The reaction time is about 1-60 h, preferably 2-48 h. Upon prolonged reaction time the guanidino-protected aminoalkyl guanidine, produced from diamines where $n=2, 3$, or 4 , i.e. diamino ethane, diamino propane or diamino
25 butane, can undergo an intramolecular cyclization forming a 5, 6, and 7-membered cyclic guanidine as a by-product. Consequently, reactions with these amines should be stopped when about 80-98%, or preferably about 85-95%, or most preferably when about 90-95% of the guanylation reagent is consumed. The reaction is easily monitored by TLC or HPLC, see the accompanying examples.

30

An important advantage of the process of the invention is the isolation of the

guanidino protected aminoalkyl guanidine by crystallization (precipitation).

According to the present invention, crystallization can occur as the product is formed or as a subsequent step after the reaction is complete by trituration with a suitable solvent or solvent mixture, preferably an hydrocarbon such as heptane. The
5 crystallization can occur in the presence or in the absence of the unreacted amine, most preferably in the presence of the unreacted diamine. Unreacted amine can be removed prior to the crystallization by vacuum distillation.

Another important advantage of the invention is the high volume efficiency with
10 which the process can produce the product, particular when using the solvent free protocol whereby a reaction mixture of 300 mL yields about 100 g of product.

The guanylation reagents are prepared by acylation of O- or S-alkylisourea or 3,5-dimethylpyrazol formamidineum nitrate using previously known methods or
15 modifications thereof, see references cited above. Some of the guanylation reagents used in the process of the invention are novel compounds per se.

According to the process of the invention, the guanidino-protected aminoalkyl guanidine is crystallized as the free base yielding the pure crude product by for
20 instance filtration or centrifugation. The product is, if necessary, purified by recrystallization by dissolution in a polar solvent such as an alcohol, for example methanol, followed by trituration with an unpolar solvent such a hydrocarbon or an alkylbenzene such as toluene or xylene. The product may optionally be precipitated and purified as a salt with a suitable acid, such as hydrochloric acid.

25

In order to illustrate but not to limit the nature of the invention and the manner of practicing the same the following examples are presented. The reactions were monitored by TLC [silica, methylene chloride/ethanol/ammonium hydroxide (aq,
25%)= 85/15/4] visualizing by either UV (254 nm) or iodine; or by HPLC (UV,
30 210 nm) using a silica column (Merck, RP select B) eluting with 30% acetonitril, 70% aqueous $\text{NH}_4\text{H}_2\text{PO}_4$ (50 mM, pH=3). NMR-spectra were recorded on a 200

MHz Bruker FT-spectrometer and the shifts (δ) are reported in ppm down field of tetramethylsilane (TMS).

Examples

5

Preparation of N-(3-aminopropyl)-N'-benzyloxycarbonyl guanidine (IIIa)

Example 1

10 To a 1000 mL round-bottomed flask was added N-benzyloxycarbonyl-O-methylisourea (II-OMe) (150 g, 0.72 mol), toluene (450 mL, 0.62 mL/mmol) and 1,3-diamino propane (Ia) (150 mL, 1.8 mol). The reaction mixture was heated to 30-40°C and stirred for 7 h followed by stirring at room temperature over night. Toluene (200 mL) was added and after 20 minutes stirring at room temperature the
15 crystals were collected by filtration. The white crystalline product was washed with toluene (4x200 mL) and dried (vacuum, 30-35°C, 8h). The yield of N-(3-aminopropyl)-N'-benzyloxycarbonyl guanidine (IIIa) was 118 g (65% in theory).
1H NMR (1M DCl/D2O): 7.28 (5H, Ph), 5.11 (2H, s, OCH₂Ph), 3.28 (2H, t, CH₂N-C), 2.92 (2H, s, CH₂NH₂), 1.86 (2H, q, C-CH₂-C). Mp: 103-105°C

20

Example 2

To a 10 mL round-bottomed flask was added N-benzyloxycarbonyl-S-methylisothiurea (II-SMe) (0.45 g, 2.0 mmol), toluene (3.5 mL) and 1,3-diamino propane
25 (Ia) (0.22 g, 3.0 mmol). The reaction mixture was heated to 40°C and stirred for 9 h followed by stirring at room temperature over night. The white crystalline product was collected by filtration, washed with toluene (2x1 mL), and dried (vacuum, 30-35°C, 4 h). The yield of N-(3-aminopropyl)-N'-benzyloxycarbonyl guanidine (IIIa) was 0.37 g (74 % in theory).

30

Example 3

N-Benzyloxycarbonyl-1-[3,5-dimethylpyrazolyl] formamidine (II-pyr) (0.55 g, 2.0 mmol) and 1,3-diamino propane (Ia) (0.37 g, 5.0 mmol) was stirred in toluene (3.5 mL) at 40°C for 5 h followed by stirring at room temperature for 1 h. Filtration, washing with toluene (2x1 mL), and drying (35°C, 15 mbar, 2 h) afforded 0.31 g (62 %) of IIIa as a white crystalline product.

Example 4

10

The method in Example 2 was applied but toluene was replaced with xylene (3.5 mL/2 mmol II-SMe) and 1.5 equiv. of diaminopropane (Ia) was employed. The reaction mixture was stirred for 8 h at 60°C and over night at 25°C. Filtration, washing (xylene), and drying gave 0.35 g (70%) of IIIa.

15

Example 5

The method in Example 1 was applied but toluene was replaced with xylene (3.5 mL/2 mmol II-OMe) and 4 equiv. of diaminopropane (Ia) was employed. The reaction mixture was stirred at 40°C for 12 h and at 25°C for 3h. Filtration, washing (toluene), and drying gave 0.31 g (62%) of IIIa.

Example 6

The method in Example 1 was applied but toluene was replaced with n-hexane (3.5 mL/2 mmol II-OMe) and 2.0 equiv. of diaminopropane (Ia) was employed. The reaction mixture was stirred at 40°C for 2 h and at 25°C for 1 h. Filtration, washing (hexane), and drying gave 0.45 g (85%) of IIIa.

30

Example 7

The method in Example 1 was applied but toluene was replaced with cyclo-hexane (3.5 mL/2 mmol II-OMe) and 2.0 equiv. of diaminopropane (Ia) was employed.

- 5 The reaction mixture was stirred at 40°C for 3 h and at 25°C for 0.5 h. Filtration, washing (cyclo-hexane), and drying gave 0.45 g (85%) of IIIa.

Example 8

- 10 The method in Example 1 was applied but toluene was replaced with acetonitrile (3.5 mL/2 mmol II-OMe) and 2.0 equiv. of diaminopropane (Ia) was employed. The reaction mixture was stirred at 40°C for 2 h, at 25°C for 1 h, and at 0°C for 0.5 h. Filtration, washing (acetonitrile), and drying gave 0.09 g (18%) of IIIa.

15 Example 9

- N'-Benzyloxycarbonyl-O-methyl-isourea (0.42 g, 2.0 mmol) and 1,3-diaminopropane (Ia) (0.37 g, 5.0 mmol) were allowed to react in isopropanol (1 mL) at 40°C. After stirring for 7 h n-hexane (3.5 mL) was added and the reaction mixture was stirred for 0.5 h at 25°C followed by filtration. The crystals were washed with hexane (2x1 mL) and dried to give 0.35 g (70 %) of IIIa.
- 20

Example 10

- 25 A mixture of N-benzyloxycarbonyl-O-methyl-isourea (II-OMe) (10.0 g, 0.048 mol) and 1,3-diaminopropane (Ia) (17.8 g, 0.240 mol) was stirred at 20°C for 18 h and at 0°C for 0.5 h. The crystalline product was collected by filtration, washed with toluene (4x15 mL), and vacuum dried (35°C, 3 h) to afford 8.2 g (68%) of white crystals of (IIIa).

Example 11

A mixture of II-OMe (0.42 g, 2.0 mmol) and Ia (0.88 g, 12 mmol) was stirred at 40°C for 2.5 h and at 25°C for 1 h followed by addition of toluene (3.5 mL). After
5 the reaction mixture had been stirred for 1 h for the crystals were collected by filtration, washed with toluene (2x1 mL), and dried (35°C, 15 mm Hg, 16h). The yield was 0.21 g (42%) of IIIa.

Example 12

10

A mixture of N-benzyloxycarbonyl-O-ethyl-isourea (II-OEt) (0.16 g, 0.7 mmol), toluene (1.3 mL) and 1,3-diaminopropane (Ia) (0.13 g, 1.7 mmol) was stirred at 40°C for 8 h and at room temperature for 48 h. The crystalline product was collected by filtration, washed with toluene (2x1 mL), and vacuum dried (35°C, 2
15 h) to afford 0.09 g (51%) of white crystals of IIIa.

Purification of N-(3-aminopropyl)-N'-benzyloxycarbonyl guanidine

20

Example 13

N-(3-Aminopropyl)-N'-benzyloxycarbonyl guanidine (IIIa) (1.0 g) was dissolved in MeOH (3 mL) and toluene (10 mL) was added. The reaction mixture was reduced (to approx. 50%) under vacuum and cooled for 1 h. The crystals were collected by
25 filtration and vacuum dried. The yield of IIIa was 0.89 g (89%).

Example 14

To a stirred solution of N-(3-aminopropyl)-N'-benzyloxycarbonyl guanidine (IIIa) (4.0 g, 16.0 mmol), 2-propanol (22 mL) and methanol (9 mL) at room temperature
30 was added over a 30 minute period HCl/2-propanol, 5 M, (13 mL, 64 mmol). The

reaction mixture was stirred at ambient temperature for 30 minutes. N-(3-Aminopropyl)-N'-benzyloxycarbonyl guanidine dihydrochloride (approximately 2 mg) was added whereby precipitation occurs immediately. The reaction mixture was stirred at ambient temperature for 2 h followed by stirring at 5°C for 1.5 h.

- 5 The crystalline product was collected by filtration, washed with 2-propanol/methanol=6/1 (2x10 mL), and vacuum dried (35°C, 2 h) to afford 4.26 g (82%) of white crystals of N-(3-aminopropyl)-N'-benzyloxycarbonyl guanidine dihydrochloride (IIIa x 2HCl).

10 **Preparation of N-(4-aminobutyl)-N'-benzyloxycarbonyl guanidine (IIIb)**

Example 15

- 15 A mixture of N-benzyloxycarbonyl-O-methyl-isourea (II-OMe) (0.42 g, 2.0 mmol), toluene (3.5 mL) and 1,4-diamino butane (Ib) (0.26 g, 3.0 mmol) was stirred at 60°C for 20 h, at 25°C for 1.5 h, and at 0°C for 2 h. The white crystalline product was filtered off, washed with toluene (2x1 mL), and dried (vacuum, 30-35°C, 2.5 h). The yield of N-(4-aminobutyl)-N'-benzyloxycarbonyl guanidine (IIIb) was 0.36 g (68% in theory).

20

¹H NMR (1M DCL/D₂O): 7.28 (5H, Ph), 5.10 (OCH₂Ph), 3.20 (2H, t, CH₂N-C), 2.87 (2H, t, CH₂-NH₂), 1.4-1.6 (4H, C-CH₂CH₂-C). Mp=110-111°C

Example 16

25

- A mixture of N-benzyloxycarbonyl-S-methyl-isothiourea (II-SMe) (0.45 g, 2.0 mmol), toluene (3.5 mL) and 1,4-diamino butane (Ib) (0.26 g, 3.0 mmol) was stirred at 40°C for 20 h and at 25°C for 12 h. The white crystalline product was filtered off, washed with toluene (2x1 mL), and dried (vacuum, 30-35°C, 2 h). The yield of N-(4-aminobutyl)-N'-benzyloxycarbonyl guanidine (IIIb) was 0.24 g (45% in theory).
- 30

Example 17

The method from example 15 was applied but toluene was replaced with xylene and the reaction mixture was stirred at 40°C for 26 h and at 25°C for 5 h.

5 Filtration, washing (xylene), and drying gave 0.36 g (68%) of IIIb.

Example 18

10 The method from example 15 was applied but the reaction was carried out neat (no solvent) and 6 equiv. of diaminobutane (Ib) was used. The reaction mixture was stirred at 40°C for 2h and at 0°C for 1 h. Filtration, washing (4x1 mL toluene), and drying gave 0.18 g (68%) of IIIb.

Preparation of different compounds of the formula III.

15

Example 19. N-(2-Aminoethyl)-N'-benzyloxycarbonyl guanidine

20 A mixture of N-benzyloxycarbonyl-O-methyl-isourea (II-OMe) (0.42 g, 2.0 mmol) and 1,2-diaminoethane (Ic) (0.72g, 12 mmol) was stirred at 40°C for 3 h and at 25°C for 1 h. The crystals were collected by filtration. The white crystalline product was washed with toluene (4x1 mL) and dried (vacuum, 30-35°C, 2h). The yield of N-(2-aminoethyl)-N'-benzyloxycarbonyl guanidine IIIc was 0.28 g (60% in theory).

25 ¹H NMR (1M DCl/D₂O): 7.28 (5H, Ph), 5.11 (2H, s, OCH₂Ph), 3.54 (2H, t, CHN-C), 3.13 (2H, t, CH₂NH₂). Mp: 106-113°C

Example 20. N-(2-Aminoethyl)-N'-benzyloxycarbonyl guanidine

30 The method in Example 19 was applied but 2.5 equiv. of diaminoethane was used and the reaction was carried out in toluene (3.5 mL/2 mmol II-OMe). The reaction

mixture was stirred 40°C for 23 h and 25°C for 1h and then filtered. Washing and drying afforded 0.32 g (68%) of IIIc.

Example 21. N-(5-Aminopentyl)-N'-benzyloxycarbonyl guanidine

5

A mixture of N-benzyloxycarbonyl-O-methyl-isourea (II-OMe) (0.42 g, 2.0 mmol), toluene (3.5 mL) and 1,5-diaminopentane (0.50, 5.0 mmol) was stirred at 40°C and for 48 h. The reaction mixture was concentrated under vacuum (35°C, 0.3 mbar) to give a crude product which was crystallized by addition of toluene followed by
10 additon of heptane. The yield of N-(5-aminopentyl)-N'-benzyloxycarbonyl guanidine (III d) was 0.3 g (60% in theory).

¹H NMR (CDCl₃): 7.3 (5H, Ph), 5.04 (2H, s, OCH₂Ph), 3.16 (2H, t, CH₂N-C), 2.62 (2H, t, CH₂NH₂), 1.3-1.4 (6H, m).

15

Example 22. N-(8-Aminooctyl)-N'-benzyloxycarbonyl guanidine

A mixture of N-benzyloxycarbonyl-O-methyl-isourea (II-OMe) (0.42 g, 2.0 mmol) and 1,8-diaminooctane (Ie) (0.72g, 12 mmol) in toluene (3.5 mL) was stirred at
20 40°C for 24 h and at 6°C for 1 h. The crystals were collected by filtration. The white crystalline product was washed with toluene (3x1 mL) and dried (vacuum, 30-35°C, 2h). The yield of N-(8-aminooctyl)-N'-benzyloxycarbonyl guanidine (IIIe) was 0.32 g (50% in theory).

25 ¹H NMR (CDCl₃):7.33 (5H, Ph), 5.06 (2H, s, OCH₂Ph), 3.03 (2H, t, CH₂-N-C), 2.64 (2H, t, CH₂-NH₂), 1.2-1.4 (12H). Mp: 108-112°C

Example 23. N-(12-Aminododecyl)-N'-benzyloxycarbonyl guanidine

30 A mixture of N-benzyloxycarbonyl-O-methyl-isourea (II-OMe) (0.42 g, 2.0 mmol), toluene (3.5 mL) and 1,12-diaminododecane (If) (1.02 g, 5.0 mmol)

was stirred at 40°C for 12 h and at 20°C for 1 h. The crystalline product was collected by filtration, washed with toluene (3x1 mL), and vacuum dried (35°C, 1.5 h) to afford 0.55 g (73%) of white crystals of N-(12-Aminododecyl)-N'-benzyloxycarbonyl guanidine (III_f).

¹H NMR (CDCl₃): 7.36-7.28 (5H, Ph), 5.08 (2H, s, OCH₂Ph), 3.07 (2H, t, J=7.0, H-1), 2.66 (2H, t, J=7.0, H-12), 1.42-1.26 (16H, m, CH₂)

Example 24. N-(3-Aminopropyl)-N'-tert-butoxycarbonyl guanidine

A mixture of tert-butoxycarbonyl-1-(3,5-dimethylpyrazolyl)-formamidine (0.38 g, 1.5 mmol) and 1,3-diaminopropane (0.56 g, 7.5 mmol) was stirred at room temperature for 17 h. The crystalline product was collected by filtration, washed with toluene (2x0.7 mL), and vacuum dried (35°C, 1.5h) to afford 0.15 g (47%) of white crystals of N-(3-aminopropyl)-N'-tert-butoxycarbonyl guanidine (III_g).

¹H NMR (CDCl₃): 3.33 (2H t, J=5.8, H-1, 2.80 (2H, t, J=6.0, H-3, 1.63 (2H, q, J=6.0, H-2), 1.44 (9H, s, CH₃ in Boc).

Preparation of guanylation reagents

Example 25. N-benzyloxycarbonyl 1-(3,5-dimethylpyrazolyl) formamidine (II-pyr)

To a stirred solution of sodium hydroxide (2.5 g, 62 mmol) and 3,5-dimethylpyrazolyl-1-formamidinium nitrate (5.0 g, 24.1 mmol) in water (60 mL) at 0°C was added over a 10 minute period benzyl chloroformate (4.35 g, 23.0 mmol). The cooling bath was removed and the reaction mixture was stirred at ambient temperature for 4.5 h. The crystalline product was collected by filtration, washed with cold water (2x20 mL), and vacuum dried

(35°C, 26 h) to afford 4.34 g (69%) of white crystals of II-pyr.

NMR (CDCl₃): 7.4-7.3 (5H, Ph), 5.95 (1H, s, C=CH-C), 5.20 (2H, s, OCH₂Ph), 2.64 (3H, s, CH₃), 2.21 (3H, s, CH₃).

5

Example 26. N-Benzyloxycarbonyl-O-ethyl-isourea (II-OEt)

A mixture of N-benzyloxycarbonyl-O-methyl-isourea (II-OMe) (0.42 g, 2.0 mmol), ethanol (10 mL) and sulphuric acid, 95% (0.33 g, 5.8 mmol) was stirred at 40°C for 14 h. The reaction mixture was filtrated, evaporated and eluated with 0, 1.25, 2.5, 5, 10, 20, 40, 80 and 100% ethylacetate/n-hexane through a silica gel column to afford 0.49 g (15%) of clear oil of II-OEt.

¹H NMR (CDCl₃): 7.40-7.30 (5H, Ph), 5.15 (2H, s, OCH₂Ph), 4.32 (2H, kv, J=7.2, OCH₂Me), 1.27 (3H, tr, J=7.1, CH₃)

15

Example 27. tert-Butoxycarbonyl-1-(3,5-dimethylpyrazolyl)-formamidine

To a stirred solution of sodium hydroxide (2.5 g, 62 mmol) and 3,5-dimethylpyrazolyl-1-formamidinium nitrate (5.0 g, 24.1 mmol) in water (60 mL) at 5°C was added over a 1 minute period di-tert-butyl-carbonate (4.78 g, 21.9 mmol). The reaction mixture was stirred 4 h at 5°C followed by stirring at room temperature over night. The reaction mixture was extracted with ethylacetate (2x25 mL), evaporated and eluated with 0, 1.25, 2.5, 5, 10, 20, 40, 80 and 100% ethylacetate/ n-hexan through a silica gel column to afford 3.31 g (62%) of clear oil of tert-butoxycarbonyl-1-(3,5-dimethylpyrazolyl)-formamidine.

25

¹H NMR (CDCl₃): 2.62 (3H, s, CH₃), 2.19 (3H, s, CH₃), 1.50 (9H, s, CH₃)

30

The best mode to carrying out the invention known at present is to use the

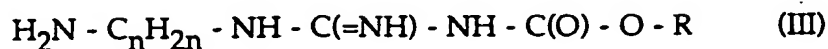
process described in Example 10.

The process of the invention is general and may be performed efficiently in one step yielding a product with high purity and in useful yields.

CLAIMS

1. A process for the production of protected ω -aminoalkyl guanidines of the general formula III, its tautomer or its salts

5



characterized in that a diamine of the general formula I

10



is reacted with a guanylation reagent of the general formula II, its tautomer or its salts

15



in which formulas $-\text{C}_n\text{H}_{2n}-$ is a linear or branched alkyl group, n is an integer 2-18;

20 R is selected from the group consisting of a linear or branched C_1 - C_{12} -alkyl group such as a lower alkyl group, and an aralkyl group such as benzyl or substituted benzyl; and

25 L is a leaving group selected from the group consisting of compounds of the general formula R^2O , R^2S , pyrazolyl and substituted pyrazolyl, where R^2 is a lower alkyl group.

2. A process according to claim 1, characterized in that $-\text{C}_n\text{H}_{2n}-$ is a linear alkyl group where n is 2-12.

30

3. A process according to claim 1, characterized in

that $-C_nH_{2n}-$ is a linear alkyl group where n is 2 - 5, 8 or 12 and R is a t-butyl or benzyl group.

4. A process according to claim 3, characterized in that
5 n is 3 and R is a benzyl group.

5. A process according to claim 1, characterized in that
said reaction is performed in the absence of or in the presence of a solvent,
preferably in the absence or if a solvent is used, preferably an aromatic
10 hydrocarbon, such as an alkylbenzene and more specifically toluene or
xylene; a hydrocarbon being linear or branched, cyclic or acyclic such as
hexane, heptane, or cyclohexane; an alkyl nitril such as acetonitril; an
alcohol such as isopropanol; or water.

15 6. A process according to claim 1, characterized in that
said reaction is performed with about at least a stoichiometric amount of
the diamine reactant, preferably 1-10 mol equivalents of diamine reactant in
relation to the guanylation reactant.

20 7. A process according to claim 6, characterized in that
the diamine reactant is used in an amount of 1.5 - 6 mol equivalents in
relation to the guanylation reactant, preferably in an amount of 2 - 4 mol
equivalents.

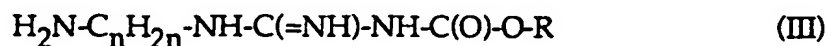
25 8. A process according to claim 1, characterized in that
said reaction is performed at a reaction temperature of approx. 20 - 80°C,
preferably at approx. 40 - 60° C.

9. A process according to claim 1, characterized in that
30 the compound III is isolated by precipitation from the reaction mixture in
form of a crystalline product.

10. A process according to claim 9, characterized in that the compound III is precipitated with an acid, preferably hydrochloric acid.

11. A compound of the general formula III, its tautomer or its salts

5



wherein $-\text{C}_n\text{H}_{2n}-$ is a linear or branched alkyl group, n is an integer from 2 to 18, and R is selected from the group consisting of a linear or branched $\text{C}_1 - \text{C}_{12}$ alkyl group such as a lower alkyl and an aralkyl group such as benzyl or substituted benzyl, with the proviso that when n=3 is R not benzyl and when n=4 is R not t-butyl or benzyl.

12. A compound according to claim 11, characterized in that $-\text{C}_n\text{H}_{2n}-$ is a linear alkyl group where n is 2-12.

13. A compound according to claim 11, characterized in that $-\text{C}_n\text{H}_{2n}-$ is a linear alkyl group where n is 2-5, 8 or 12 and R is a t-butyl or benzyl group.

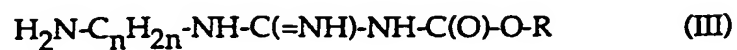
20

14. N-(3-Aminopropyl)-N'-t-butyloxycarbonyl guanidine,
N-(2-Aminoethyl)-N'-benzyloxycarbonyl guanidine,
N-(5-Aminopentyl)-N'-benzyloxycarbonyl guanidine,
N-(8-Aminooctyl)-N'-benzyloxycarbonyl guanidine, and
25 N-(12-Aminododecyl)-N'-benzyloxycarbonyl guanidine.

15. N-Benzyloxycarbonyl-1-[3,5-dimethylpyrazolyl] formamidine and t-butoxycarbonyl-1-(3,5-dimethylpyrazolyl)-formamidine.

30

16. A product of the formula III, its tautomer or its salts



5

wherein $-\text{C}_n\text{H}_{2n}$ is a linear or branched alkyl group, n is an integer 2-18
and R is selected from the group consisting of a linear or branched $\text{C}_1 - \text{C}_{12}$
alkyl group such as a lower alkyl, and an aralkyl group such as benzyl or
substituted benzyl produced by a process defined in one of claims 1-9.

10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 94/00517

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C07C 279/24, C07C 277/08, C07D 231/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: C07C, C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE, A, 463576 (SCHERING-KAHLBAUM AKT.-GES.), 31 July 1928 (31.07.28) --	1-10
X	EP, A2, 0277626 (THE ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND), 10 August 1988 (10.08.88), Example I, page 10, line 1 - line 19; Example V, page 11, line 20 - line 33 --	11-14,16
X	GB, A, 2085444 (RICHTER GEDEON VEGYESZETI GYAR RT), 28 April 1982 (28.04.82), page 4, line 46 - line 59; page 3, line 42 - page 4, line 8 --	11-14,16

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

& document member of the same patent family

Date of the actual completion of the international search

30 August 1994

Date of mailing of the international search report

08-09-1994

Name and mailing address of the ISA/

Swedish Patent Office

Box 5055, S-102 42 STOCKHOLM

Facsimile No. +46 8 666 02 86

Authorized officer

Gerd Strandell

Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 94/00517

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL SOCIETY, Perkin transactions II, Volume 15, 1973, (London), Anthony F. Hegarty et al, "Kinetics and Mechanism of Hydrolysis of the Amidinating Agent 1-(N1-Benzoylamidino)-3, 5-dimethylpyrazole" page 2054 - page 2060 --	15
X	FR, A1, 2294699 (BOTTU), 16 July 1976 (16.07.76), the claims; page 3, line 21, line 23 --	15
A	ORGANIC PREPARATIONS AND PROCEDURES INT., Volume 20, No 5, 1988, Barbara Rzeszotarska et al, "Arginine, histidine and tryptophan in peptide synthesis. The guanidino function of arginine", page 427 - page 464, see page 455 --	15
A	INT. J. PEPTIDE PROTEIN RES., Volume 37, 1991, Zhenping Tian et al, "Guanidination of a peptide side chain amino group on a solid support" page 425 - page 429 -- -----	1-16

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/07/94

International application No.

PCT/SE 94/00517

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-A- 463576	31/07/28	NONE	
EP-A2- 0277626	10/08/88	AU-B- 614734 AU-A- 1120388 JP-A- 63201198 US-A- 4914189	12/09/91 11/08/88 19/08/88 03/04/90
GB-A- 2085444	28/04/82	AT-B- 384228 AU-B- 535688 AU-A- 6672581 BE-A- 887224 CA-A- 1158641 DE-A- 3108810 FR-A,B- 2491463 JP-A- 57064653 NL-A- 8100391 SE-B,C- 452326 SE-A- 8100302 SU-A- 1178322	12/10/87 29/03/84 22/04/82 27/07/81 13/12/83 19/05/82 09/04/82 19/04/82 03/05/82 23/11/87 08/04/82 07/09/85
FR-A1- 2294699	16/07/76	DE-A- 2557514 GB-A- 1513280 JP-A- 51125282	01/07/76 07/06/78 01/11/76